

Western blot results for NKA-isoforms and FXYD1 in type-I and type-II human skeletal muscle fibre pools before and after a single exercise session.

Protein abundance was analysed in type I and II fibres from vastus lateralis in the control (CON-leg) and blood flow-restricted leg (BFR-leg) at rest before (Rest) and immediately after (Exh) an intense exercise session (single-leg, knee-extensor exercise at 90% peak power output) to exhaustion. BFR was induced by inflation of an occlusion cuff around the most proximal portion of the leg. Data were analysed for significance (p<0.05) using a two-way RM ANOVA with time and treatment (CON vs BFR) as factors. Data are means ± 95% confidence intervals.

Note the sample size as indicated. The low sample size in A and B is due to the analyses still being undertaken in the lab. The full data set is therefore not available. n=8 for FXYD1 as blots for two subjects could not be validly quantified due to noise on the image.

As evidenced on the figure (A to F), there were no differences between rest and exhaustion for all isoforms in both legs.

**In conclusion**, there is no likely effect of an acute exercise session on NKA-isoform protein content in both fibre types.

## Murphy et al. (J Physiol, 2004):

"Intense exercise up-regulates Na+,K+-ATPase (NKA) isoform mRNA, but not protein expression in human skeletal muscle"

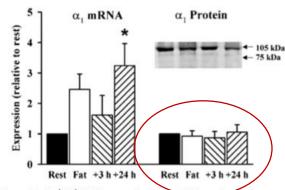


Figure 3. Na $^+$ ,K $^+$ -ATPase  $\alpha_1$  isoform mRNA and protein expression before and after acute one-legged knee extensor exercise

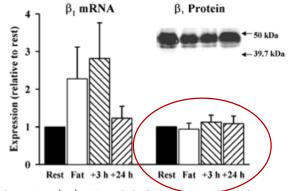


Figure 6. Na $^+$ ,K $^+$ -ATPase  $\beta_1$  isoform mRNA and protein expression before and after acute one-legged knee extensor exercise

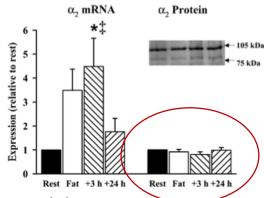


Figure 4. Na $^+$ ,K $^+$ -ATPase  $\alpha_2$  isoform mRNA and protein expression before and after acute one-legged knee extensor exercise

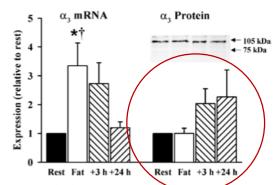


Figure 5. Na<sup>+</sup>,K<sup>+</sup>-ATPase  $\alpha_3$  isoform mRNA and protein expression before and after acute one-legged knee extensor exercise

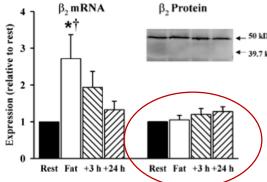


Figure 7. Na $^+$ ,K $^+$ -ATPase  $\beta_2$  isoform mRNA and protein expression before and after acute one-legged knee extensor exercise

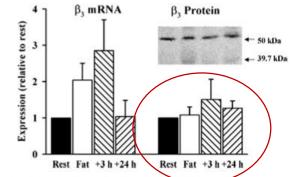


Figure 8. Na<sup>+</sup>,K<sup>+</sup>-ATPase  $\beta_3$  isoform mRNA and protein expression before and after acute one-legged knee extensor exercise

## Confirmation of our fibre type-specific observations in whole-muscle homogenates of human skeletal muscle:

In agreement with our findings in type I and II fibre pools (previous image/page), there was no effect of a single exercise session (40% peak power output to exhaustion) on NKA-isoform protein abundance in whole-muscle homogenate of human vastus lateralis in the study by Murphy and colleagues (red circles). Note that data are expressed as means ± SEM (n=15), making the error bars relatively small (vs. 95%CI or SD).

In conclusion, NKA-isoform protein content is unlikely to change significantly with an acute exercise session in human skeletal muscle – neither in fibre type-specific nor in fibre type-heterogeneous samples.

It can be questioned whether NKA  $\alpha 3$  protein may be upregulated more rapidly than other isoforms (Fig. 5, Murphy et al. 2004). However, the very low abundance of this isoform in human muscle, along with the great intersubject variability (several folds) in its protein content (and the non-significant gains in this study by Murphy), both questions the time-course of protein translation and downplays the physiological impact of this isoform in human skeletal muscle (Christiansen et al., 2017, DOI: 10.1101/151035, BioRxiv).